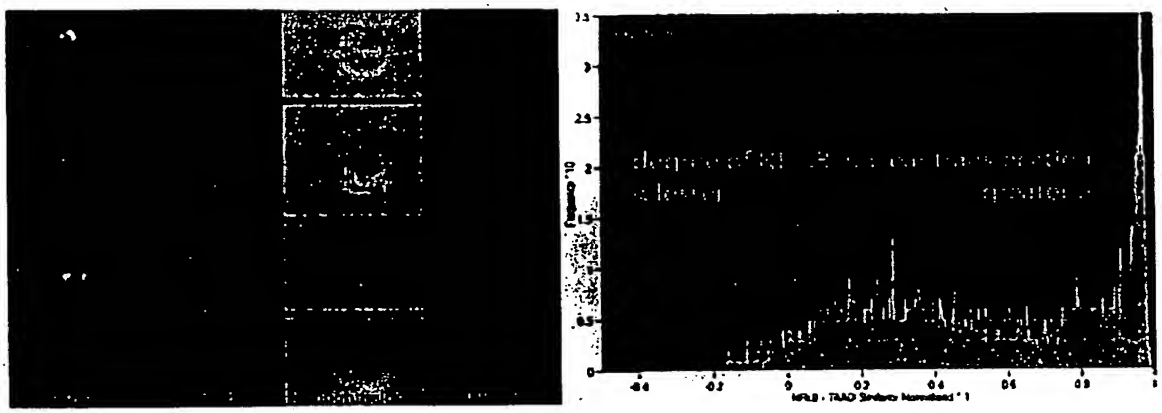


Figure 1. NF-kB Nuclear Translocation in Immune Cells
The data (above left) show cells imaged simultaneously in darkfield, green fluorescence, brightfield, and red fluorescence. The sample consisted of a monocytic cell line stained with an antibody against the NF-kB transcription factor (green) as well as a nuclear stain (red). Cells treated with lipo-polysaccharide (image rows 2-4) exhibit translocation of NF-kB from the cytoplasm to the nucleus while untreated cells lack NF-kB in the nuclear compartment (top row). A statistical analysis of imagery from 6616 cells quantitatively characterizes the degree of NF-kB nuclear translocation in the sample. Amnis' ImageStream platform is the only cell analysis technology that can perform this valuable assay on immune cells in suspension.

Fig. 1

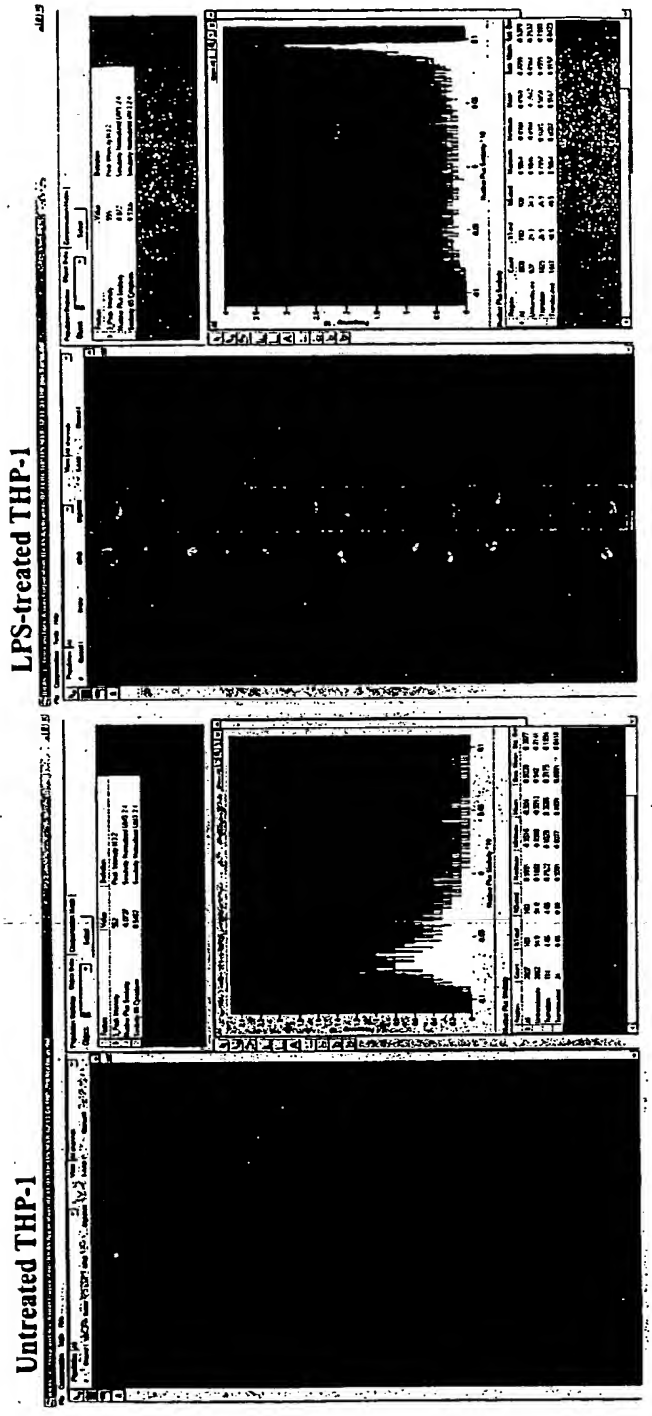
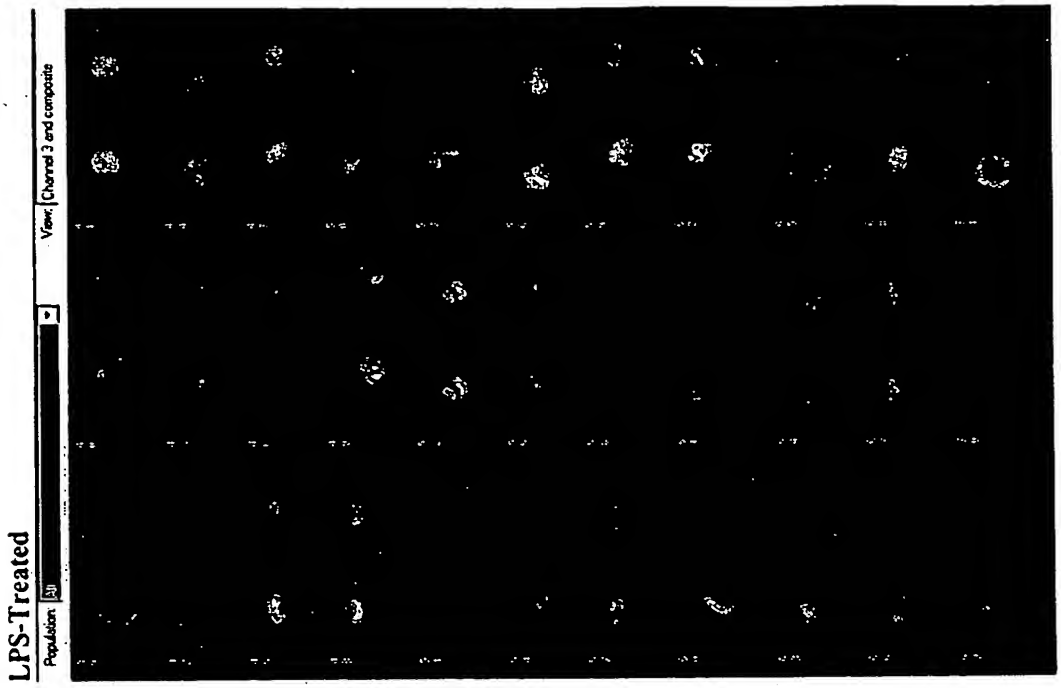


Fig. 2


LPS-Treated
Population
All
View:
Channel 3 and composite

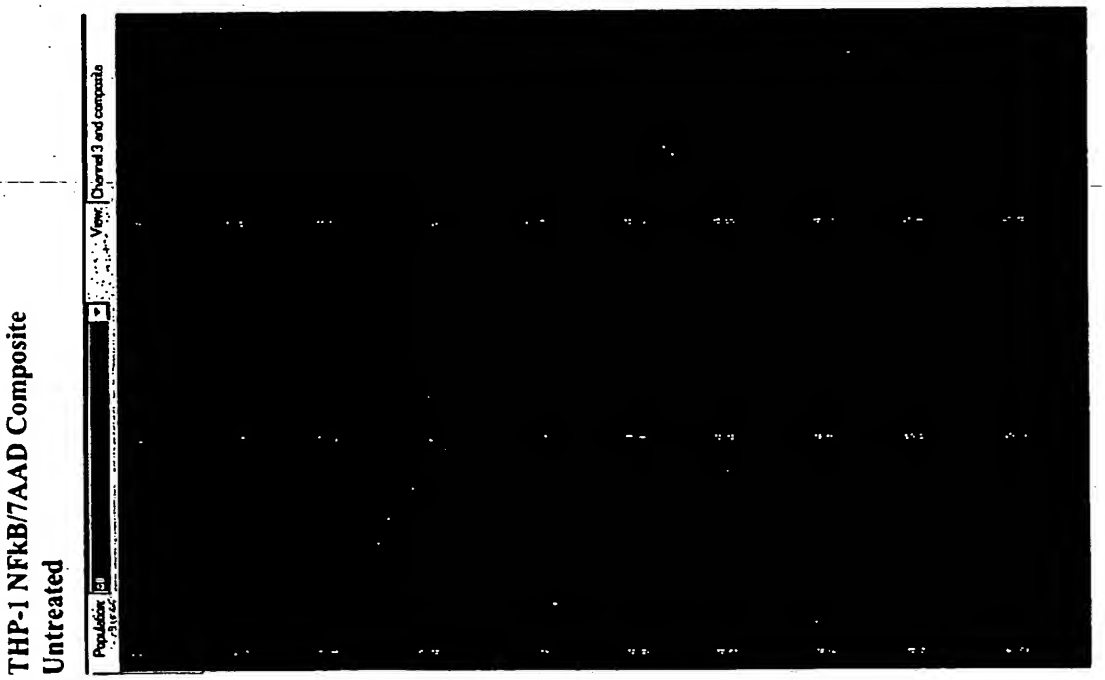

THP-1 NFkB/7AAD Composite
Untreated
Population
All
View:
Channel 3 and composite

Fig. 3

Object 8:

7-AAD stain

MASKS: 85% UM3 75% UM5 Standard M5

NFκB stain

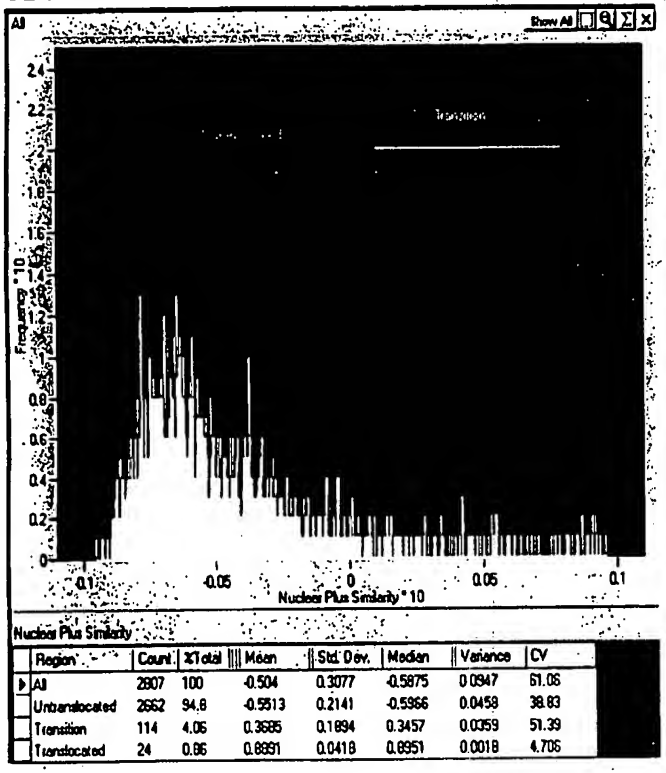
MASKS: 85% UM3 75% UM5

	Feature	Value	Definition
▶	3_Peak Intensity	571	Peak Intensity M3 2
	Nuclear Plus Similarity	-0.155	Similarity Normalized UM5 2 4
	Similarity 85 Cytoplasm	0.5003	Similarity Normalized UM3 2 4

Fig. 4

COMPARTMENTAL CORELATION FEATURE:

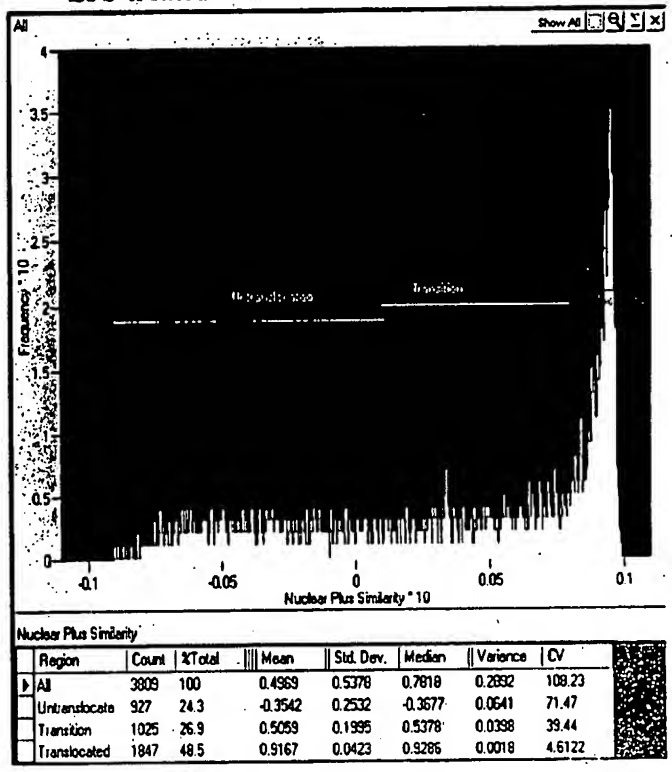
Untreated



Nuclear Plus Similarity

Region	Count	%Total	Mean	Std. Dev.	Median	Variance	CV
All	2607	100	-0.504	0.3077	-0.5875	0.0947	61.06
Untranslocated	2662	94.8	-0.5513	0.2141	-0.5966	0.0459	38.83
Transition	114	4.06	0.3685	0.1894	0.3457	0.0359	51.39
Translocated	24	0.86	0.8891	0.0418	0.8951	0.0018	4.706

LPS-treated



Nuclear Plus Similarity

Region	Count	%Total	Mean	Std. Dev.	Median	Variance	CV
All	3809	100	0.4969	0.5378	0.7818	0.2892	108.23
Untranslocate	927	24.3	-0.3542	0.2532	-0.3577	0.0641	71.47
Transition	1025	26.9	0.5059	0.1995	0.5378	0.0398	39.44
Translocated	1847	48.5	0.9167	0.0423	0.9286	0.0018	4.6122

Median Compartmental Corelation Feature:

Untranslocated = -0.5966 +/- 0.2141

Translocated = 0.9286 +/- 0.0423

Difference of 1.5252

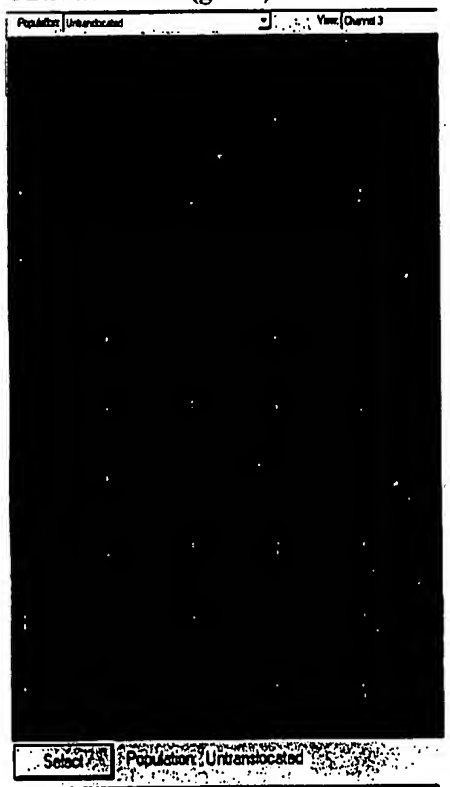
Fig. 5

Express Mail No.: EV336655140US
Title: IMAGE-BASED QUANTITATION OF MOLECULAR TRANSLOCATION
Inventors: Thaddeus C. George et al.
Serial No.
Docket No.: 120122.406P1

Select | Population: All

Feature	Mean	Median	Std. Dev.
3_Peak Intensity	517.76	513	80.18
Nuclear Plus Similarity	-0.504	-0.5875	0.3077
Similarity 85 Cytoplas	0.2683	0.2576	0.2089

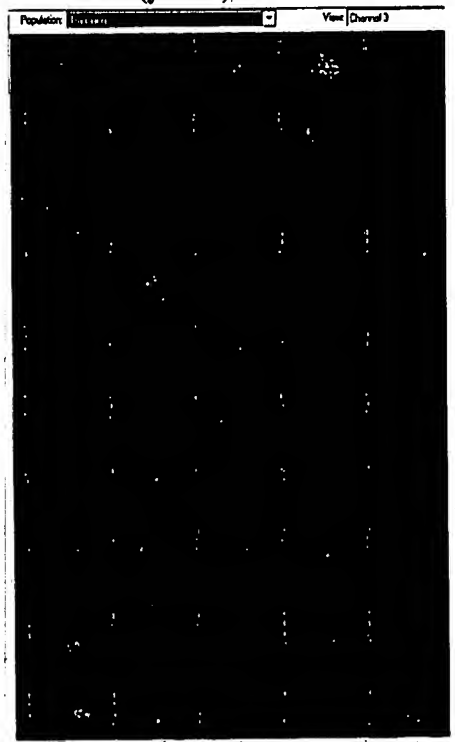
Untranslocated (green)



Select | Population: Untranslocated

Feature	Mean	Median	Std. Dev.
3_Peak Intensity	516.82	513	77.09
Nuclear Plus Similarity	-0.5513	-0.5966	0.2141
Similarity 85 Cytoplas	0.2455	0.2446	0.1817

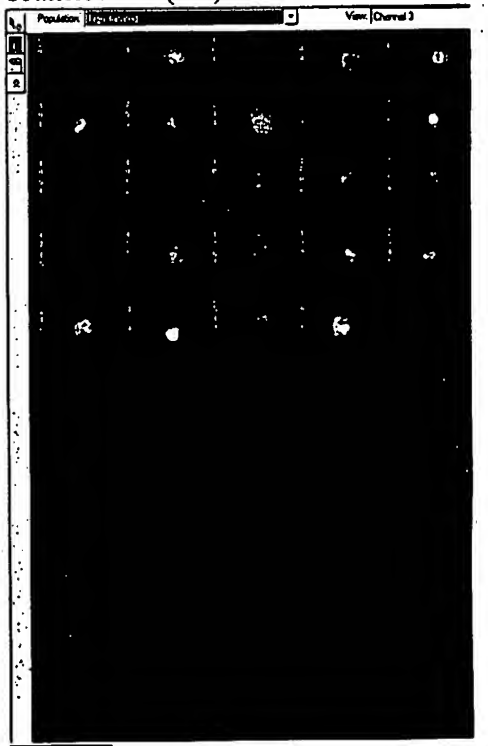
Transition (yellow)



Select | Population: Transition

Feature	Mean	Median	Std. Dev.
3_Peak Intensity	516.48	505	95.28
Nuclear Plus Similarity	0.3685	0.3457	0.1894
Similarity 85 Cytoplas	0.6939	0.7159	0.1279

Translocated (red)



Select | Population: Translocated

Feature	Mean	Median	Std. Dev.
3_Peak Intensity	626.33	627.5	195.17
Nuclear Plus Similarity	0.8891	0.8951	0.0418
Similarity 85 Cytoplas	0.9259	0.9373	0.0398

Fig. 6A

Select | Population: All

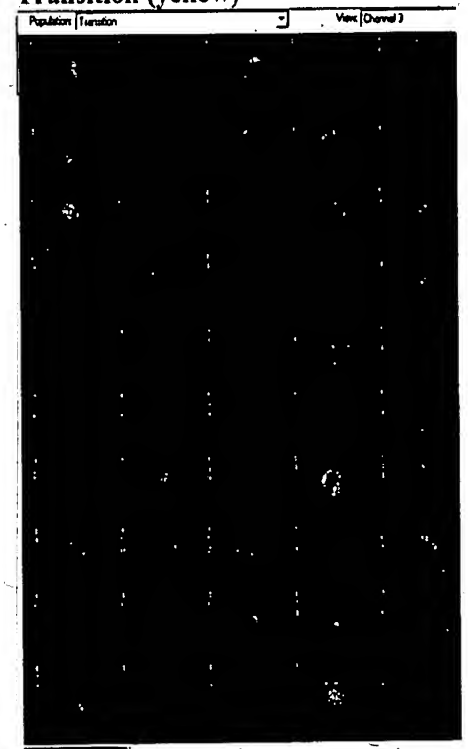
	Feature	Mean	Median	Std. Dev.
▶	3_Peak Intensity	673.63	656	125.11
	Nuclear Plus Similarity	0.4969	0.7818	0.5378
	Similarity 85 Cytoplas	0.7687	0.8684	0.2373

Untranslocated (green)

Select | Population: Untranslocated

	Feature	Mean	Median	Std. Dev.
▶	3_Peak Intensity	622.25	617	92.23
	Nuclear Plus Similarity	-0.3542	-0.3677	0.2532
	Similarity 85 Cytoplas	0.4128	0.4393	0.1805

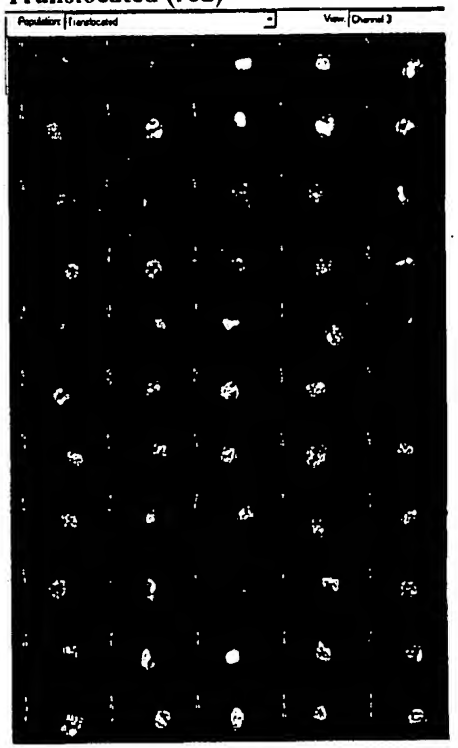
Transition (yellow)



Select | Population: Transition

	Feature	Mean	Median	Std. Dev.
▶	3_Peak Intensity	593.99	601	88.66
	Nuclear Plus Similarity	0.5059	0.5378	0.1995
	Similarity 85 Cytoplas	0.7693	0.7827	0.0829

Translocated (red)



Select | Population: Translocated

	Feature	Mean	Median	Std. Dev.
▶	3_Peak Intensity	740.45	737	121.81
	Nuclear Plus Similarity	0.9167	0.9286	0.0423
	Similarity 85 Cytoplas	0.946	0.9547	0.0307

Fig. 6B

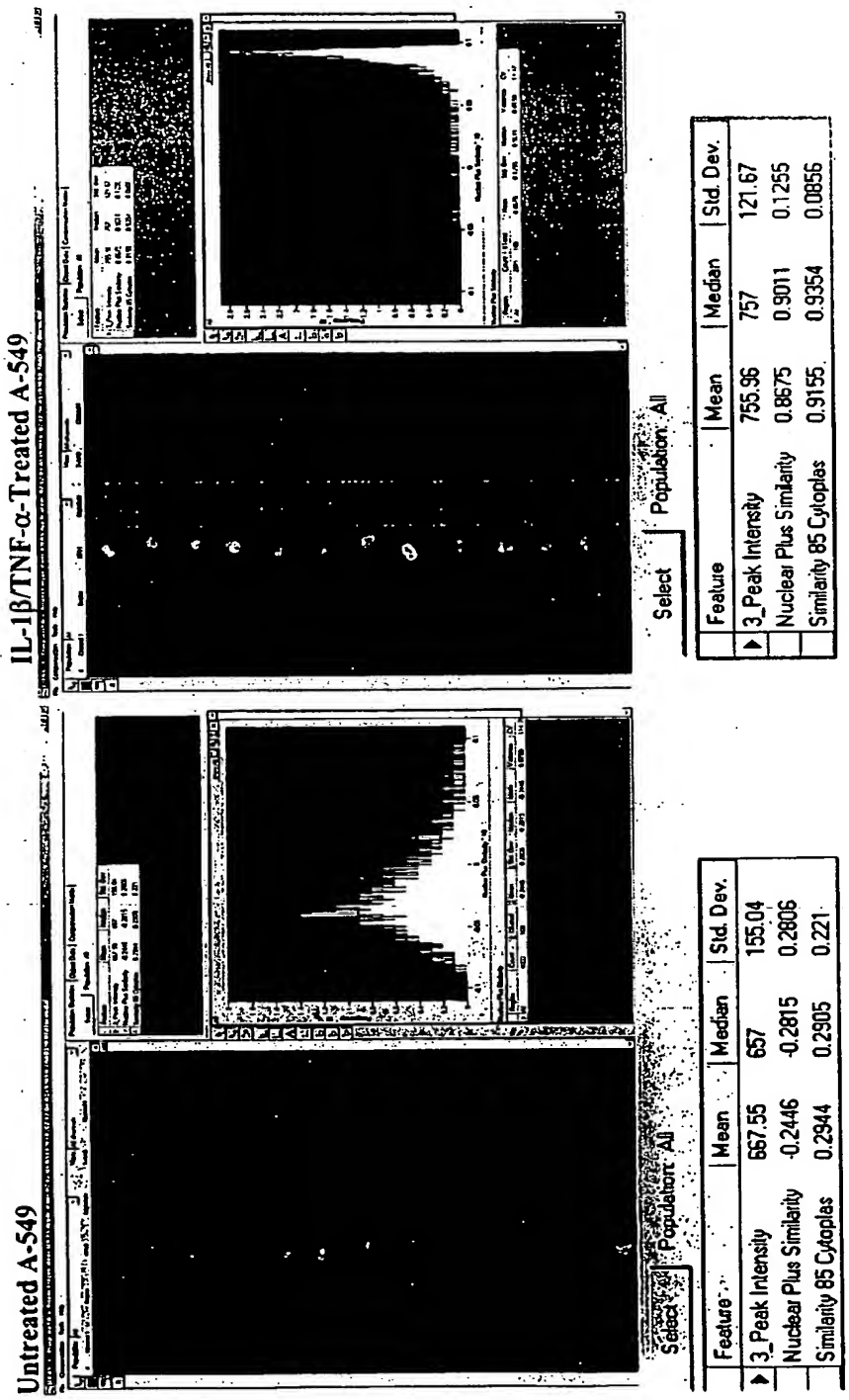
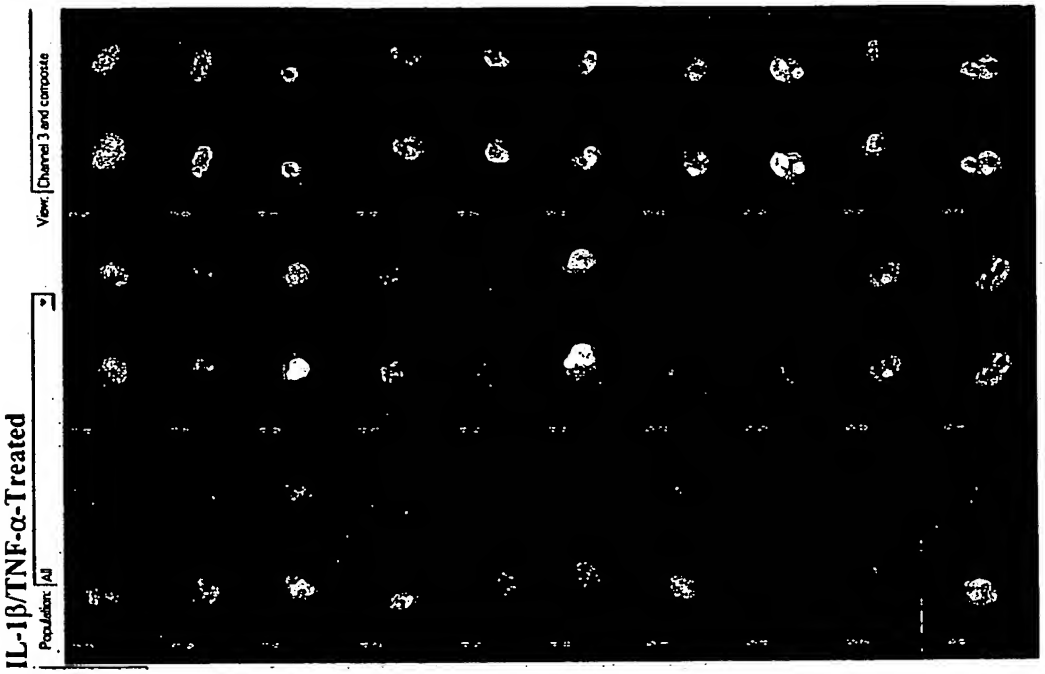

Untreated A-549
IL-1β/TNF-α-Treated A-549
Select
Population: All
Feature | Mean | Median | Std. Dev.
3_Peak Intensity | 667.55 | 657 | 155.04
Nuclear Plus Similarity | -0.2446 | -0.2815 | 0.2806
Similarity 85 Cytoplas | 0.2944 | 0.2905 | 0.221
Feature | Mean | Median | Std. Dev.
3_Peak Intensity | 755.96 | 757 | 121.67
Nuclear Plus Similarity | 0.8675 | 0.9011 | 0.1255
Similarity 85 Cytoplas | 0.9155 | 0.9354 | 0.0856

Fig. 7


IL-1β/TNF-α-Treated
Population: All
View: Channel 3 and composite

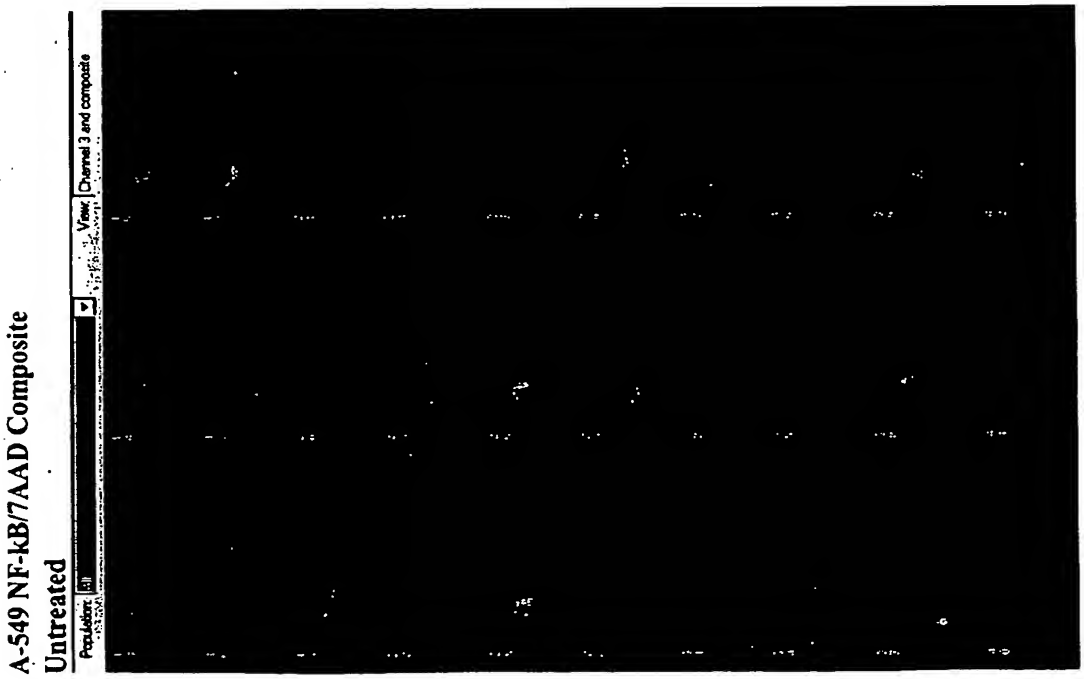
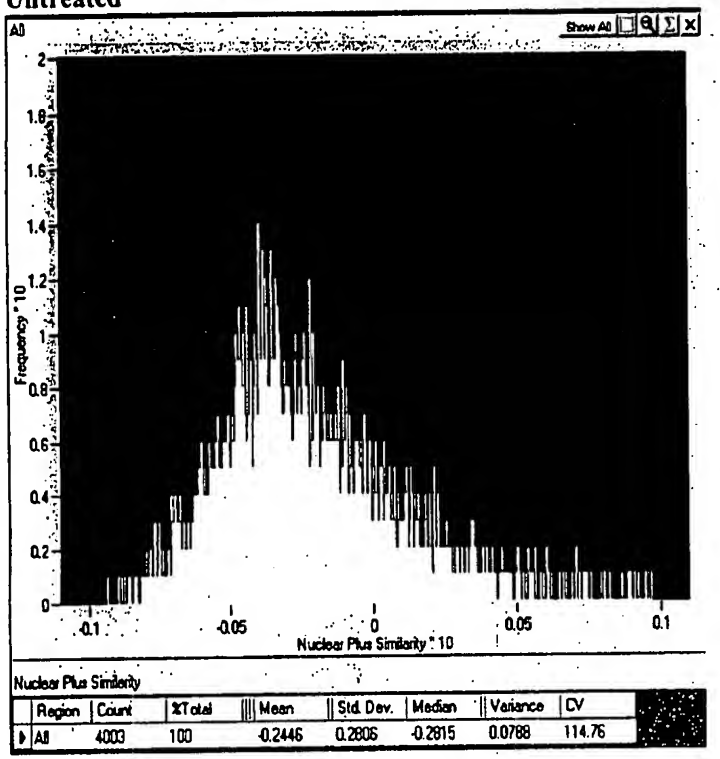

A-549 NF-kB/7AAD Composite
Untreated
Population: All
View: Channel 3 and composite

Fig. 8

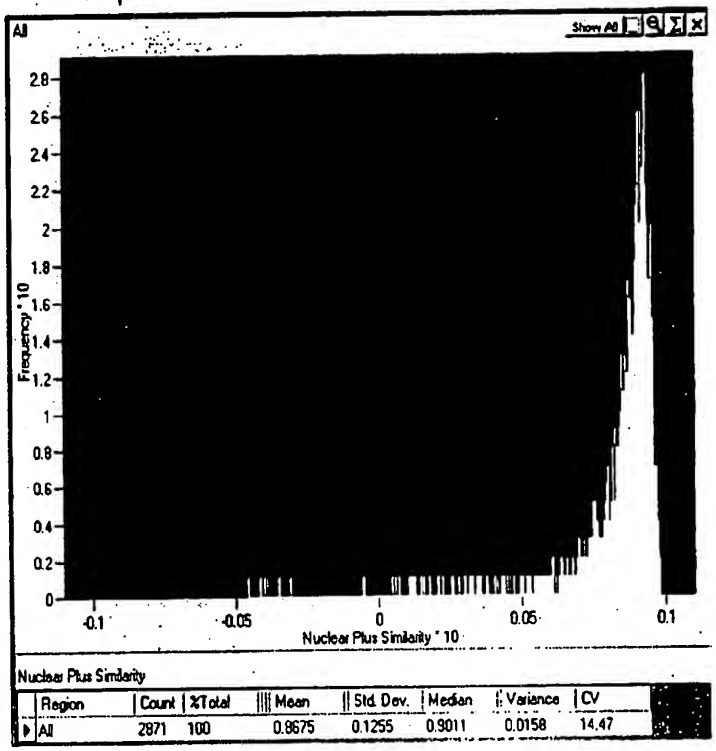
Compartmental Corelation Feature: A-549 Cells

Untreated



Region	Count	%Total	Mean	Std. Dev.	Median	Variance	CV
All	4003	100	-0.2446	0.2806	-0.2815	0.0788	114.76

IL-1β/TNF-α-Treated



Region	Count	%Total	Mean	Std. Dev.	Median	Variance	CV
All	2871	100	0.9675	0.1255	0.9011	0.0158	14.47

Median Compartmental Corelation Feature:

-2815 +/- 0.2806

0.9011 +/- 0.1255

Difference of 1.1826

Fig. 9

Express Mail No.: EV336655140US
Title: IMAGE-BASED QUANTITATION OF MOLECULAR TRANSLOCATION
Inventors: Thaddeus C. George et al. Serial No. Docket No.: 120122.406P1